Effects of body position and extension of the neck and extremities on lung volume measured via computed tomography in red-eared slider turtles (Trachemys scripta elegans)

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Objective—To determine the effects of body position and extension of the neck and extremities on CT measurements of ventilated lung volume in red-eared slider turtles (Trachemys scripta elegans).

Design—Prospective crossover-design study.

Animals—14 adult red-eared slider turtles.

Procedures—CT was performed on turtles in horizontal ventral recumbent and vertical left lateral recumbent, right lateral recumbent, and caudal recumbent body positions. In sedated turtles, evaluations were performed in horizontal ventral recumbent body position with and without extension of the neck and extremities. Lung volumes were estimated from helical CT images with commercial software. Effects of body position, extremity and neck extension, sedation, body weight, and sex on lung volume were analyzed.

Results—Mean ± SD volume of dependent lung tissue was significantly decreased in vertical left lateral (18.97 ± 16.63 mL), right lateral (24.59 ± 19.16 mL), and caudal (9.23 ± 12.13 mL) recumbent positions, compared with the same region for turtles in horizontal ventral recumbency (48.52 ± 20.08 mL, 50.66 ± 18.08 mL, and 31.95 ± 15.69 mL, respectively). Total lung volume did not differ among positions because of compensatory increases in nondependent lung tissue. Extension of the extremities and neck significantly increased total lung volume (127.94 ± 35.63 mL), compared with that in turtles with the head, neck, and extremities withdrawn into the shell (103.24 ± 40.13 mL).

Conclusions and Clinical Relevance—Vertical positioning of red-eared sliders significantly affected lung volumes and could potentially affect interpretation of radiographs obtained in these positions. Extension of the extremities and neck resulted in the greatest total lung volume. (J Am Vet Med Assoc 2013;243:1190–1196)
and craniocaudal projections. For evaluation of the lower respiratory tract, the craniocaudal projection is considered most useful because no superimposition with visceral organs occurs and the left and right lungs can be evaluated separately.

To obtain diagnostic radiographs of the lungs in cheloniens, it has been anecdotally recommended that a horizontal body position and horizontal radiographic beam orientation is superior for craniocaudal and lateral radiographic views, compared with a vertical body position and vertical beam orientation. It has been suggested that in a vertical body position, coelomic organs are displaced and compress the lungs, leading to an artificially decreased lung volume, which could create errors in radiographic interpretation. However, conversion of a standard radiographic unit for horizontal beam orientation is time-consuming and not technically possible with all machines. Thus, despite the preference for horizontal body positions during radiography, vertical body positions are still commonly used to obtain craniocaudal and laterolateral radiographic projections.

It has also been reported that withdrawal of the neck, head, and extremities into the shell reduces lung volume and causes superimposition, making radiographic interpretation more challenging. Placing cheloniens in horizontal body position on a wooden block or plastic container has been recommended to achieve immobilization and extension of the extremities and neck. However, in semiaquatic or shy cheloniens, this technique is usually not possible because these animals often withdraw their extremities and head into the shell. Therefore, sedation or general anesthesia can be required to achieve sufficient immobilization, which allows for extension and fixation of the extremities and neck. The paucity of controlled studies investigating the effects of body position, extension or withdrawal of the neck and extremities, and chemical restraint on lung morphology in radiographic images makes it difficult to provide guidelines for appropriate radiographic imaging techniques for evaluation of the lower respiratory tract in cheloniens. Therefore, the objective of the study reported here was to investigate and quantify the effects of body position (vertical vs horizontal) and extremity and neck position (extended vs withdrawn) on ventilated lung volume measured via CT in conscious and sedated male and female red-eared slider turtles (Trachemys scripta elegans). We hypothesized that these variables would have a significant effect on ventilated lung volume in this species.

**Materials and Methods**

**Animals**—Fourteen university-owned adult red-eared slider turtles (8 males and 6 females) were included in this study. Mean ± SD body weight was 0.7 ± 0.1 kg (1.5 ± 0.2 lb) for males and 1.0 ± 0.1 kg (2.2 ± 0.2 lb) for females. Mean ± SD carapacial length was 18.7 ± 0.6 cm for males and 20.2 ± 0.5 cm for females. All turtles were presumed healthy on the basis of results of physical examination. After acquisition, turtles were acclimatized to the housing conditions for ≥ 8 weeks prior to the study. Each turtle was treated with cefazidime (25 mg/kg [11.4 mg/lb], SC, q 72 h for 5 to 7 injections) and fenbendazole (30 mg/kg [13.6 mg/lb], PO, q 14 days for 2 treatments) because of a prior history of subclinical bacterial and intestinal nematode infections in other animals received from the same supplier. The turtles were housed in 1,800-L open tanks (5 to 10 turtles/tank), in which they had access to dechlorinated water for swimming and dry areas for basking. Air temperature was set at 27° to 28°C, with light provided for 14 h/d. Turtles were fed commercially available floating food sticks 3 to 4 times/wk. On each experimental day, animals were transported to the institution’s radiology suite and held in transport containers without water access at room temperature (24° to 25°C) for 8 hours before experiments. The use of animals for this study was approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison School of Veterinary Medicine.

**Experiments**—To accurately determine lung volumes of the turtles, whole body CT scans were performed under 6 conditions (Figure 1). For evaluation of conscious turtles, carapace openings were blocked with tape after the extremities, neck, and head were withdrawn into the shell. Scans were performed with the turtles in horizontal ventral recumbent, vertical caudal recumbent, vertical left lateral recumbent, and vertical right lateral recumbent body positions. Imaging of sedated turtles was performed in the horizontal ventral recumbent body position, once with the extremities, neck, and head inside the taped shell and once with extremities and neck fixed in extended positions. Waterproof medical tape was used for fixation of the neck and extremities in extended as well as flexed positions.

Sedation and chemical restraint, which allowed for extension of the neck and extremities, were induced by SC administration of dexmedetomidine (0.1 mg/kg [0.045 mg/lb]), midazolam (1.0 mg/kg [0.45 mg/lb]), and ketamine (2.0 mg/kg [0.91 mg/lb]). The CT scans were performed 45 to 60 minutes after drug administration. All turtles received flumazenil (0.05 mg/kg [0.023 mg/lb]) and atipamezole (1.0 mg/kg) SC to reverse the effects of midazolam and dexmedetomidine after completion of the CT scans.

Experiments were performed on 2 days, 14 days apart. On the first day, CT scans were performed for conscious turtles in the described horizontal and vertical body positions. Fourteen days later, CT scans of conscious turtles in the horizontal ventral recumbent position were repeated to establish baseline for lung volumes specific for that experimental day. Then turtles were sedated and placed in the horizontal ventral recumbent body position with the extremities, head, and neck withdrawn, and a CT scan was performed to assess for an effect of sedation. Next, the extremities and necks of sedated turtles were extended and taped in position and the CT scan was repeated (with turtles still in horizontal ventral recumbent body position) to assess for an effect of extension of the extremes and neck (Figure 1). The sequence in which the effects of various vertical body positions were evaluated was not randomized because several animals in the same body position were imaged simultaneously via CT. The vertical left lateral recumbent, caudal recumbent, and right lateral recumbent body positions were evaluated in that order. In an effort to avoid...
carryover effects of the previous vertical position on lung volume (ie, the effect of shifted coelomic organs during previous positioning), turtles were positioned for 15 minutes in the horizontal ventral recumbent body position after each vertical positioning. The duration of each CT scan was approximately 1 to 2 minutes, and turtles were monitored before each CT scan and afterward while kept in horizontal body position.

**CT imaging**—A single slice helical CT unit was used for all evaluations. Whole body scans were performed in helical scan mode with a 2-mm slice thickness and 1-mm reconstruction interval. A medium- (detail) and high-frequency (bone) image reconstruction algorithm was used. Further technical parameters applied included the following: display field of view, 50 cm; tube rotation time, 0.8 seconds; peak voltage, 120 kV; and current, 130 mA. Up to 9 turtles were imaged simultaneously.

Computed tomographic images were transferred to a radiation therapy planning station for volumetric estimates of ventilated lung tissue. With an autocontour threshold of 800 to 4,096 Hounsfield units, the left and right lungs were outlined as operator-defined regions of interest in every slice. The left, right, and total lung volume for each turtle was calculated for each of the conditions evaluated with manufacturer-supplied software. To examine the effect of the vertical caudal recumbent body position, the ventilated lung volume present in the cranial and caudal halves (50% of the total measured carapacial length) was also calculated. Three-dimensional reconstruction of CT images was performed for visualization purposes (Figure 2).

**Data analysis**—For data analysis, commercially available software was used. The data were tested for normality via a Shapiro-Wilk test. A 2-tailed Student t test was used to compare body weight and carapacial length between females and males. Because both variables were significantly different between sexes, the lung volume data were adjusted for body weight and carapacial length to test for differences in lung volume dependent on sex and its interactions with other conditions tested. Two-way repeated-measures ANOVA was used to evaluate the data for the effects of sex, body position, and extremity and neck extension on partial and total lung volume. A Tukey test was used for post hoc pairwise comparisons if significant differences were found between groups. A paired 2-tailed Student t test was used to compare lung volumes between turtles with and without sedation. A value of P < 0.05 was considered significant. The desired statistical power of performed tests was >0.8 to avoid the possibility of a type 2 statistical error. The data were expressed as mean ± SD. Lung volume of turtles in each of the conditions evaluated was compared with the baseline value of same-day results for conscious turtles in the horizontal ventral recumbent body position with the extremities, head, and neck withdrawn inside the taped shell. The lung volume for each condition was then expressed as mean ± SD as well as mean ± SD percentage of the baseline value (calculated on the basis of each individual turtle’s percentage difference from baseline).

**Results**

Ventilated lung volumes determined by use of CT were summarized for conscious turtles in various body positions with the neck, head, and extremities withdrawn in the shell and for sedated turtles in the horizontal ventral recumbent position with and without extension of the extremities and neck (Table 1). Body...
position had a significant \((P < 0.001)\) effect on lung volume. The volume of the dependent lung in vertical lateral recumbent positions was significantly \((P < 0.001)\) decreased and that of the nondependent lung was significantly increased, compared with baseline volumes for turtles in the horizontal ventral recumbent position.

In the vertical caudal recumbent position, lung volume in the caudal 50% of the carapacial length was significantly decreased and that in the cranial 50% was significantly \((P < 0.001)\) increased, compared with values for the same region in the horizontal ventral recumbent body position.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Left lung</th>
<th>Right lung</th>
<th>Cranial 50% of CL</th>
<th>Caudal 50% of CL</th>
</tr>
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<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
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<tr>
<td>Body position</td>
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<tr>
<td>Horizontal ventral recumbent*</td>
<td>99.18 ± 38.02</td>
<td>48.52 ± 20.08</td>
<td>50.66 ± 18.08</td>
<td>67.23 ± 25.01</td>
<td>31.95 ± 15.69</td>
</tr>
<tr>
<td>Vertical left lateral recumbent</td>
<td>91.48 ± 36.33</td>
<td>18.97 ± 14.65</td>
<td>72.51 ± 25.78</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Vertical right lateral recumbent</td>
<td>101.45 ± 38.71</td>
<td>76.86 ± 23.43</td>
<td>24.59 ± 19.16</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Vertical caudal recumbent</td>
<td>104.02 ± 34.81</td>
<td>53.61 ± 18.78</td>
<td>50.41 ± 19.16</td>
<td>94.8 ± 27.28</td>
<td>9.23 ± 12.13</td>
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<tr>
<td>Experiment 2</td>
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<tr>
<td>Nonsedated turtles (head, neck, and extremities withdrawn)†</td>
<td>103.24 ± 40.13</td>
<td>50.94 ± 20.42</td>
<td>52.30 ± 19.93</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sedated turtles</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Head, neck, and extremities withdrawn</td>
<td>88.06 ± 37.93</td>
<td>43.62 ± 19.42</td>
<td>44.44 ± 18.73</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Head, neck, and extremities extended</td>
<td>127.94 ± 35.53</td>
<td>64.60 ± 18.12</td>
<td>63.34 ± 17.56</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

For experiment 1, all turtles were conscious and had the head, neck, and extremities withdrawn into the shell and carapace openings secured with medical tape. For experiment 2, all turtles were in the horizontal ventral recumbent position; sedation and chemical restraint were provided with dexmedetomidine\(^1\) (0.1 mg/kg), midazolam\(^2\) (1.0 mg/kg), and ketamine\(^3\) (2.0 mg/kg) SC. Percentages reflect the volume relative to the baseline value for the same measurement for each experiment and were calculated on the basis of each animal’s percentage difference from baseline.

\(^*\)Position in which baseline lung volumes were measured for comparisons among body positions in conscious turtles. \(^†\)Condition in which baseline lung volumes were measured for evaluation of effects of sedation and extension of the head, neck, and extremities.

CL = Carapacial length. ND = Not determined.

\(^1\)Volume is significantly \((P < 0.01)\) different from the baseline value for the same measurement.
In sedated turtles, extension of the extremities and neck resulted in a significant \( (P < 0.01) \) increase in ventilated lung volumes. The factors of sedation \( (P = 0.239; \text{power} = 0.21 \text{ for total lung volume}) \) or sex \( (P = 0.57; \text{power} = 0.05 \text{ for total lung volume}) \) had no significant effect on lung volume.

**Discussion**

The lungs in red-eared slider turtles are capable of undergoing substantial volume changes with changes in body position and extension or flexion (withdrawal into the shell) of the extremities and neck. Several anatomic features result in the flexibility and compressibility of turtle lungs, including their simple sac-like structure with little parenchyma, lack of a well-developed bronchial tree, and lack of a diaphragm.\(^4\) The cranial and caudal portions of the lungs are thin-walled, simple membranous sacs, which undergo changes in volume owing to contraction of the shoulder and pelvic muscles, which are involved in the physiologic respiratory cycle in turtles.\(^4\) During compression of peripheral portions of the lungs, air is forced through the central, less compressible portions of the lungs, in which most gas exchange occurs.\(^4\) Changes in lung volume can also be caused by several physiologic factors all leading to differences in filling of the coelomic cavity, including changes in coelomic pressure, gastrointestinal filling, and presence of ovarian follicles and eggs.\(^20\) The results of the present study show that the physiologic adaptability of the lungs predisposes turtles to iatrogenic changes in ventilated lung volume if vertical body positions are used for radiographic imaging.

Changes in partial lung volume were apparent in all 3 evaluated vertical body positions. Because of the mobility of the coelomic viscera and direct attachment of the stomach and liver to the lungs via the postpulmonary septum,\(^4\) these changes are gravity dependent. Similar gravity-dependent changes in lung volume have been reported in red-tailed hawks (Buteo jamaicensis).\(^21\) The vertical left lateral and vertical right lateral recumbent body positions resulted in a significant reduction of the dependent lung volume and a compensatory increase in the nondependent lung volume in the turtles of our study. In the vertical caudal recumbent body position, a significant reduction of the lung volume was observed within the caudal 50% of the carapace, which was compensated for by a significant increase in lung volume in the cranial 50% of the carapace. These compensatory changes in partial lung volume explain why total lung volume did not change significantly in any of the body positions tested.

During vertical positioning of the turtles, compression of the lungs by intracoelomic organs caused a significant reduction of the dependent lung volume, and such changes may affect a clinician’s ability to detect focal or unilateral abnormalities in dependent lung areas. A similar phenomenon has been shown in other animals positioned in vertical lateral recumbency for thoracic radiographic imaging.\(^15-16\) For example, compression of the dependent lung tissue and loss of ventilation, with subsequent image contrast reduction, have been reported as an underlying cause for obscuring of focal pulmonary lesions because of border effacement (silhouette effect) in dogs and humans.\(^16-18\) However, in dogs positioned in lateral recumbency, no volume change in the nondependent lung occurs, and hence the total lung volume is reduced.\(^23\) In contrast, turtles used in the present study had no significant change in total lung volume, despite compression of the dependent lung, because of volume expansion in the nondependent lung.

In this study, the sequence in which vertical body positions were evaluated was not in a random order because several turtles underwent CT examination simultaneously. Although turtles were placed in the horizontal ventral recumbent body position for 15 minutes between the different vertical body positions, the possibility of a carryover effect of the previous position cannot be entirely ruled out because it is unknown whether a 15-minute interval was sufficient to eliminate this effect. However, in the vertical caudal recumbent position, which was evaluated after the vertical left lateral recumbent position, volumes of the left and right lungs were not significantly different from each other. Therefore, a clinically relevant carryover effect, which could have been avoided by randomization, seems less likely.

Increased expansion of the nondependent lung might potentially increase the diagnostic value of radiographic imaging performed with turtles in vertical body positions because hyperexpansion might allow easier identification of focal or unilateral lung lesions in nondependent lung tissue. If vertical lateral recumbent body positions are used for radiographic evaluation of lower respiratory tract disease in turtles, we recommend that imaging be performed in both left and right lateral recumbent positions for radiographic evaluation of turtles with lower respiratory tract disease.

Although it has been recommended that the extremities of cheloniens should be extended for radiographic imaging, the effect on lung volume has not been previously investigated.\(^17-18\) In our study, total lung volume was significantly increased in sedated turtles with necks and extremities completely extended, compared with values for the same turtles when fully withdrawn into the shell. This finding is explained by space occupation of the extremities, head, and neck during withdrawal into the shell. Extension of the extremities and neck contributes to increased negative coelomic pressure, leading to expansion of the lungs and inhalation of air.\(^\)\(^3\) In some species of tortoises, it might be possible to achieve extension of the extremities by simply placing the animal on a block to suspend it off of the examination surface. Terrestrial tortoises will usually drop their extremities as a consequence, and radiographs can be obtained with the beam in horizontal or vertical position. However, in most freshwater semi-aquatic turtles, such as red-eared slider turtles, suspension on a block will usually result in withdrawal into the shell.
which can be considered as a predator avoidance behavior. As a clinical consequence, these species usually require chemical restraint to facilitate extension of the extremities and neck for radiographic imaging. In compromised or depressed turtles, it might be possible to achieve extension of the neck and extremities without the use of chemical restraint. The chemical restraint protocol used in this study was partially reversible with antagonists available for midazolam and dexmedetomidine. Administration of a benzodiazepine and \( \alpha_2 \)-adrenoreceptor agonist allowed for substantial reduction of the ketamine dose. This protocol achieved deep sedation of all turtles, sufficient to perform the extension of the neck and extremities and the CT scans. Sedation is currently underutilized in reptile anesthesia; our results indicate that sedation is sufficient for extension of the extremities and neck, and general anesthesia is not needed for optimal radiographic positioning of turtles. In healthy turtles used in the present study, sedation had no significant effect on lung volume; however, the statistical power of the test was too low, and therefore further research is necessary to investigate the effects of sedation on lung volume in turtles. It is currently unknown whether other chemical restraint protocols, in particular general anesthesia protocols (eg, propofol) that result in dose-dependent apnea and complete muscle relaxation, have a possible effect on lung volume in turtles. If follow-up radiography is performed in clinical settings for reevaluation of the lower respiratory tract, the same chemical restraint protocol should be used for both evaluations to avoid possible changes in lung volume secondary to different chemical restraint protocols.

Turtles of both sexes were included in this study. No significant difference in total lung volume was detected between sexes, even after correction for body weight and carapacial length, and no significant interaction between sex and body position was detected. All turtles in the study were considered adults on the basis of body size and weight. However, the reproductive status of the turtles was not determined. Because we performed CT scans for several turtles simultaneously, differentiation between inspiration and expiration was not possible. Red-eared slider turtles, a semiaquatic species, breathe episodically in clusters, followed by a period of apnea that varies in length. Therefore, it is likely that most turtles were in the expiratory phase of respiration during CT scans. In addition, the extremities, head, and neck were withdrawn and carapace openings were secured with tape for most of the imaging procedures performed. In this position, because of the physiology of respiration in chelonians, complete inspiration would not be possible.

Results of the present study provide evidence that placing red-eared slider turtles in various vertical body positions can significantly affect partial lung volumes. Therefore, we recommend horizontal radiographic positioning of chelonian patients to avoid compression of lung tissue by shifting coelomic organs, which could increase the risk of radiographic misinterpretation and misidentification of focal or unilateral lung disease. If vertical positioning and radiographic beam orientation cannot be avoided for laterolateral projections, then vertical right and left lateral recumbent views should both be obtained for evaluation of each lung in the nondependent state. We also recommend that the neck and extremities be extended for imaging if possible to increase lung inflation and therefore potentially improve the diagnostic value of imaging of turtles with diseases of the lower respiratory tract. Further research is necessary to determine whether the observed effects of positioning and sedation on partial and total lung volumes in red-eared slider turtles also occur in other chelonian species.

References